EXHIBIT 2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

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MEMORANDUM

Subject: Technical Support Document for the Response to the Emergency Citizen Petition Seeking Suspension of Registration for Clothianidin Based on Claims of Imminent Hazard to the Environment.

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This technical support document (TSD) provides EFED's analysis of the claims and the scientific literature cited in support of the allegation of "imminent hazard" in the Emergency Citizen Petition (dated March 20, 2012) submitted by the Center for Food Safety and International Center for Technology Assessment concerning the pesticide clothianidin. The TSD is organized by summarizing each of the main arguments made in the petition and its supporting Appendix B (State of the Science Report) that address the claim of imminent hazard that clothianidin poses to the environment and then providing EFED's response to each argument.

EFED also provides an analysis of the specific studies cited to support the petitioner's arguments. Typically EFED evaluates studies from the open literature consistent with the

Open Literature Guidance (USEPA, 2011) and classifies studies from the open literature that pass the screening process as either "Invalid" or of "Quantitative" or "Qualitative Use" utility in risk assessments. While the formal open literature screening process used by the EPA Office of Research and Development to support the ECOTOX database and the additional evaluation tools used by OPP to review open literature will be conducted at the time of the Registration Review risk assessment, EFED has considered the studies cited in the petition and made a preliminary conclusion that none of these studies contain sufficient detail that would enable the quantitative use of these studies for the clothianidin risk assessment. However, several of the studies appear likely to be considered of Qualitative value for the risk assessment and EFED has attempted to expedite the evaluation process for these studies by further characterizing them as of low, medium or high qualitative use. For the studies determined to be of medium or high qualitative use, EPA will complete Data Evaluation Records (DERs) according to the Open Literature Guidance (USEPA, 2011) and place these reviews in the docket at the time of the full petition response.[1]

Executive Summary:

EFED has evaluated the arguments and information that the petitioners use to support the claim of imminent hazard to the environment posed by use of clothianidin. EFED agrees with the petitioner that clothianidin use is widespread and that under certain environmental conditions the compound can persist. EFED also agrees with the petitioners that clothianidin can exhibit both acute and sub-lethal toxic effects at different concentrations. The incident reports and literature data show that acute, lethal exposures of foraging honey bees to clothianidin are possible. However, the sporadic and limited nature of the incident data indicates that conditions resulting in acute exposures of bees to clothianidin are not typically widespread or common and are not likely to result in the loss of entire colonies or diminished populations of honey bees over large geographic areas.

The petitioners also claim that the available data indicate that chronic exposures of bees to clothianidin residues in contaminated pollen and nectar is possible and that other neonicotinoid pesticides can have sub-lethal effects on bees including effects on mobility, feeding activity, memory and learning capacity at high enough doses. However, EFED believes that the available residue data indicate that typical concentrations of neonicotinoid pesticides in pollen and nectar are generally significantly below the levels at which these effects occur.

The petitioners also claim that clothianidin and other neonicotinoid pesticides can interact synergistically both with other pesticides and with pathogens, increasing the toxicity of clothianidin to honey bees and the susceptibility of honey bees to infection. EFED does

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[1] Although the response to the Emergency Petition did not allow time for the full review traditionally conducted in connection with registration or registration review, EFED was able to review the cited studies in an expedited, but adequate manner to assess the claims cited in the petition and its supporting documents.

not agree that the available data indicate that clothianidin and other nitroguanidine neonicotinoids have synergistic effects with other pesticides as the only study on synergism the petitioners cited indicated synergism of cyano-substituted neonicotinoids and fungicides, but the same study tested for and did not indicate synergism of nitroguanidine neonicotinoids with fungicides. Nitroguanidine neonicotinoids would also not be expected to act synergistically due to their lack of degradation by the honeybee P450 enzymes. Clothianidin is a nitroguanidine neonicotinoid pesticide, not a cyano-substituted neonicotinoid.

The data cited by the petitioners on synergism of nitroguanidine neonicotinoids and pathogens (*i.e. Nosema*) is inconclusive; the relationship between imidacloprid treatment levels and Nosema spore loads did not appear to result in a concentration dependent response. Also, while spore loads were increased in individual bees, whole colonies treated with the same amounts of imidacloprid did not exhibit increased levels of the gut parasite. Additionally, the effects seen were generally at concentrations above what the petitioners' data indicates that honey bees would typically be exposed to in contaminated pollen or nectar.

The petitioners also imply that clothianidin and other nitroguanidine neonicotinoids are correlated with and in part responsible for honey bee declines and Colony Collapse Disorder (CCD). However, EFED is not aware of any data indicating that honey bee declines or the incidence of CCD in the U.S. is correlated with the use of pesticides in general or with the use of neonicotinoids in particular. Honeybee colony declines in the U.S. have been reported for more than 60 years (vanEngelsdorp and Meixner, 2010) while clothianidin and other nitroguanidine neonicotinoid pesticides have been registered for a relatively short period of time. Elevated residues of clothianidin and other nitroguanidine neonicotinoids have not been found in appreciable amounts in colonies that have suffered from CCD and the occurrence of CCD is not higher in regions of the country where clothianidin and other nitroguanidine neonicotinoids are most frequently used.

Finally, the open literature studies cited by the petitioners represent a broad array of methods and measurement endpoints. Many of the methods used in the laboratory and field studies cited involved novel approaches which did not account for potential confounding effects. In addition, the vast majority of the biological effects data cited by the petitioner were studies conducted on imidacloprid. There is some uncertainty with associating endpoints from these studies, especially for chronic sub-lethal effects with equivalent doses of clothianidin. Studies conducted on other chemicals, such as pyrethroids, acaricides or cyano-substituted neonicotinoids, introduce further uncertainty. Also, many of the measurement endpoints reported in the studies involved sublethal effects to individual bees and for which there were no clear linkages with assessment endpoints of impaired survival, growth or reproduction at the level of the whole colony. As such, EPA considers the utility of the studies in terms of their ability to either qualitatively or quantitatively define a predictive causal relationship between clothianidin and the individual honeybee or, more importantly, the bee colony, to be low.

EFED's analysis of Petitioner's Claims

A. The petition alleges that research indicates honey bee colony declines and other native bee declines are linked to the combination of systemic pesticides, pathogens, and nutrition

EFED Response: After review of the information related to this claim, and the uncertainty surrounding this complex scientific issue, EFED finds that the information cited in the petition does not provide adequate evidence to show the potential for substantial adverse effects of nitroguanidine neonicotinoid insecticides on insect pollinators, in general, and on honeybees, specifically. EFED further finds that the available data (including incident reports) do not indicate that nitroguanidine neonicotinoids, including clothianidin in particular, are causing substantial adverse effects on bees that would result in the loss of large numbers of honeybee colonies across the United States.

EFED acknowledges that the currently available incident data indicate the use of clothianidin in the absence of a sticking agent for seed treatment, in combination with dry windy conditions, can generate and mobilize fugitive dust in sufficient quantities to result in acute mortality events. However, this kind of incident has the potential to occur with any insecticide applied as a seed-treatment whenever treated seeds are planted and weather conditions are conducive to the movement of dusts.

In addition to the limited number of incidents that raise issues related to sticking agents, EPA has recently received new incident data from several states and Canada following the Spring, 2012 corn planting. These data, pending review and confirmatory residue analysis, may inform future risk assessments, regulatory decisions, and assist in identifying measures to mitigate acute exposures of bees and other pollinators. However, our preliminary review of these incidents indicates that these incidents do not support the conclusion that use of clothianidin is the cause of colony loss and declining national populations of honey bees, as asserted by the petition. Rather, the preliminary analysis indicates that, while dead bees are found in these incidents, there were no colony losses in these incidents and regardless of whether these incidents were caused by clothianidin residues or other causes, they do not by themselves indicate consistent, adverse effects on bees that are resulting in the loss of large numbers of honeybee colonies across the United States.

B. The petition alleges that neonicotinoid pesticide use is ubiquitous and that this ubiquitous use and the general persistence of neonicotinoids results in long-term exposures. The petitioners state that all but 0.2% of corn is treated with neonicotinoids, primarily clothianidin and thiamethoxam.

*EFED Response: The Agency agrees with the petitioner's claim that clothianidin use is widespread and common. Clothianidin is a member of the nitroguanadine class of neonicotinoids. As of 2010, the combined percentage of the U.S. corn crop treated with nitroguanadine neonicotinoid pesticides (*i.e., *clothianidin, thiamethoxam, imidacloprid,*

and dinotefuran combined) is a little less than 90% with clothianidin responsible for a majority of neonicotinoid seed treatment applications to corn.

EFED's evaluation of clothianidin finds that the chemical is persistent across a wide range of soil conditions and also in aquatic environments under conditions of reduced or low sunlight (aerobic soil metabolism half lives of 148—1155 days, aerobic aquatic metabolism half life of approximately 180 days MRIDs 45422326, 45422327, 45422328, 46826903), but there is no evidence that clothianidin persists in pollen and nectar at concentrations that would likely cause adverse effects on honeybee populations. In addition to the information already submitted by the registrant and reviewed by EFED, the registrant is currently conducting additional studies in California, requested by the California Department of Pesticide Regulation, that examine residues in soils and plants after multiple-year applications to determine 1) if clothianidin residues accumulate in pollen and nectar following the compound's use at a site in preceding years and 2) whether residues are higher than concentrations after a single year's application. The preliminary results of those studies do not demonstrate a significant cumulative effect of successive treatment years on residues in pollen/nectar (Beedle and Harbin, 2011).

As support for their claim, petitioners cited studies by Bonmatin et al. 2003 and 2005, and Krupke et al. 2012 as evidence of the presence and accumulation of neonicotinoids in soils and expression in pollen and nectar. Bonmatin et al. 2003 reported that soils exposed for one or two years previously through imidacloprid seed treatments had imidacloprid detections in 97% of these soils. Of these detections, 19% were above the LOD[2] (0.1 µg a.i./kg), but below the LOQ[3] (1.0 µg a.i./kg) and 78% were above the LOQ. The study authors reported a mean concentration of 6 µg a.i./kg soil in the detections that were above the LOQ. Bonmatin also reported that sunflower capitules from untreated sunflowers growing in these contaminated soils contained an average of 1.5 µg imidacloprid/kg . Imidacloprid was detected in 83% of pollen samples (LOD = 0.3 µg a.i./kg) from seed treated sunflowers with 58% of pollens having concentrations above the LOQ (1.0 µg a.i./kg) with an average of 3 µg a.i./kg (range 1.0—11.0 µg a.i./kg). In a very small sample (n=5), imidacloprid was also detected in 80% of pollen samples from seed treated maize with the positive samples containing an average of 2 µg/kg (range 1-3 µg/kg). Krupke et al. 2012 reported concentrations of clothianidin in soils of up to 6.3 ppb for maize and 9.6 for soy and reported concentrations in corn pollen up to 3.9 ppb.

The Agency has not established a formal honeybee colony level NOAEC[4] for imidacloprid or other pesticides; however the mean residues in pollen reported in

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2 LOD refers to the Limit of Detection and means the minimum level at which the particular pesticide's residues can be distinguished from the absence of those residues within a stated confidence limit.

3 LOQ refers to the Limit of Quantification and means the minimum level at which the concentration of a particular pesticide can be determined within a stated confidence limit.

4 NOAEC refers to the No Observable Adverse Effects Concentration

Bonmatin et al. and Krupke et al. are generally more than an order of magnitude below levels showing any effects that the Agency is aware of from either the petitioners' cited studies or the broader scientific literature and submitted data on sublethal effects, either to individual bees or to whole colonies. An exception to this is the Colin et al. article that the petitioners cite in which the number of "active" bees at 6 µg a.i./kg imidacloprid treatment decreased. This exposure level is above levels typically encountered in pollen and nectar, but would be much closer to the levels reported by Bonmatin et al. and Krupke et al. As described below in its analysis under point H, however, EFED has serious concerns regarding using this study quantitatively or even qualitatively due to previous control data pooled with controls concurrently run in the experiment, lack of replication in the controls that were concurrently run in the experiment, an unbalanced test design, lack of ability to define a dose-response due to only one treatment concentration tested, unclear definition of "active" bees, concerns about how this parameter was observed and validated, and lack of measurement of food consumption by the bees.

Additionally, the petitioner cites Bonmatin et al. 2005 as evidence of accumulation of imidacloprid in soils over multiple years. EFED disagrees with petitioners that this study is evidence of the accumulation in soil over multiple years. Even though the study authors state that a "slight accumulation phenomenon in soils cannot be excluded," the study does not conclusively state or provide evidence that such a phenomenon is actually occurring. Additionally, the distance between untreated and treated fields was not reported in all of these studies, creating some uncertainty as to whether detected concentrations in soil and pollen are due to contaminated dust drift from abraded seeds or due to plant uptake of the chemical, which had persisted in the soil, and the subsequent systemic expression of the chemical in the plant's pollen and nectar.

The following section is EFED's analysis of the cited studies in support of the claim that neonicotinoids show a long term exposure concern.

Bonmatin JM, Marchand PA, Carvet R, Moineau I, Bengsch ER, and ME Colin. 2003. Method for analysis of imidacloprid in soils, plants and pollens. Analytical Chemistry 75(9): 2027-2033.

The study authors reported that soils treated one or two years previously with imidacloprid seed treatments had imidacloprid concentrations >1 µg/kg in 78% (and detections in 97%) of soils with a mean concentration of 6 µg/kg in soils above the LOQ and 19% of soils above the LOD (0.1 µg a.i./kg), but below the LOQ (1.0 µg a.i./kg). Sunflower capitules from untreated sunflowers growing in contaminated soils were reported to contain average imidacloprid concentrations of 1.5 µg/kg (sample size was not indicated). Imidacloprid was detected in 83% of pollen samples from treated sunflowers with 58% of the samples having an average of 3 µg/kg. These average values are below the levels at which biological effects were seen in any of the cited studies by the petitioners. The authors reported that 5 pollen samples from maize were analyzed with four of the samples containing imidacloprid at an average concentration of 2 µg/kg (range 1-3 µg/kg). The study authors did not report the distance between untreated areas

and other, treated areas; therefore uncertainty exists as to whether imidacloprid concentrations found on soils which were not treated during the current year were due to the persistence of imidacloprid treatments from a prior year, or whether contaminated dust from abraded seed may have drifted from untreated areas to treated areas. At this time, EFED believes this study would likely be considered of medium qualitative use for clothianidin risk assessment purposes due to small sample sizes for the maize pollen analysis and because the study was conducted on imidacloprid; there is uncertainty in estimating clothianidin residues based on imidacloprid residue data. EFED anticipates having a full review and finalized DER of this study by the time EPA responds to the other claims in the petition.

Bonmatin JM, Moineau I, Charvetet R, Colin ME, Fleche C, and ER Bengsch. 2005. Behaviour of imidacloprid in Fields. Toxicity for Honey Bees. Environmental Chemistry: Green Chemistry and Pollutants in Ecosystems; Springer-Verglag Berlin Heidelberg, 483-494.

EFED was unable to determine whether this study was peer-reviewed or if peer reviewed, at what level, since this is a chapter in a book rather than a published journal article. Some of the results reported here were previously described in Bonmartin et al. 2003. The study authors report imidacloprid detections in 91% of non-organically farmed soils (n = 62). On currently seed treated land, 9 out of 10 soils sampled had an average concentration of 12 µg a.i./kg (range 2-22 µg/kg) . Imidacloprid was reported to be present on 97% of soils that had no seed-treated plants for one year, but had been treated one or two years previously. Soils that had not been directly exposed for one year had a mean value of imidacloprid of 6 µg/kg compared to 8 µg/kg for soils that had not been directly exposed for two years. The study authors reported mean concentrations of imidacloprid of 8 µg/kg in treated sunflower capitulums when imidacloprid was applied at 0.7 mg/seed. The amount of imidacloprid actually available to honeybees in sunflower pollen and nectar was unclear from these results and may be somewhat less than the concentration reported for the entire flower head. The authors also reported in this article that out of 29 pollen samples from untreated sunflower crops, two had positive detections, but were below the LOQ. EFED does not typically use books as references in risk assessment due to their typical lack of extensive peer review. Further, residues were not measured in pollen and nectar and the study was conducted on imidacloprid and its utility in estimating clothianidin residues on sunflowers is limited. Therefore, this study would likely be considered of low qualitative use for clothianidin risk assessment purposes.

C. The petitioners allege that clothianidin has been observed to cause the type of bee kills attributed to Colony Collapse Disorder (CCD)

EFED Response: EFED finds that the petitioner did not provide data that demonstrates exposure to clothianidin results in effects on honey bee colonies that are consistent with those associated with Colony Collapse Disorder (CCD). CCD is characterized by the complete loss of adult forage bees without any signs of bee mortality leaving colonies with ample brood and food reserves along with a small cluster of hive-bees including the

queen (vanEngelsdorp et al, 2009a and vanEngelsdorp and Meixner, 2009). While clothianidin was attributed to a bee kill incident in Germany in 2008 where 11,000 colonies were affected and several subsequent bee kills of considerably lower magnitude in other countries within the European Union in 2011, these incidents have been acute mortality events where dead bees were clearly evident. The characteristics of these clothianidin-related incidents are not consistent with the characteristics of CCD.

D. The petitioners imply that European experience with neonicotinoid use, bee kills and the European suspensions of neonicotinoids and the resulting lack of bee kills and lack of CCD following the suspensions indicate that neonicotinoids are causing CCD.

EFED Response: At this time, clothianidin is suspended from use as a seed treatment on maize and sunflower in France and for maize in Germany and Italy. The incidence of CCD in the EU has not been well characterized; however, to EPA's knowledge, the incidence of CCD and bee pollinator declines in general in the EU have not been correlated with the use of neonicotinoids in general or with clothianidin in particular. High overwintering colony losses prior to clothianidin suspension in Germany also have not been correlated with clothianidin or other nitroguanidine neonicotinoid residues in bee bread (Genersch et al. 2010). An absence of CCD in countries that have suspended some uses of neonicotinoids in Europe by itself would not implicate neonicotinoid pesticides as the cause of CCD. Mass bee kills such as those in Germany in 2008 and Slovenia in 2011 were events that included acute exposures to abraded dust from clothianidin seed treatments. As noted in point C, CCD is characterized by the complete loss of adult forage bees without any signs of bee mortality, leaving colonies with ample brood and food reserves along with a small cluster of hive-bees including the queen. The acute mass bee mortality incidents reported in Germany and Slovenia appear to be completely different events from CCD-related events. Additional mass acute bee poisonings on the scale of these incidents have not been reported in Germany since it suspended clothianidin maize seed treatment.

E. The petitioners claim that bees are regularly exposed to levels of clothianidin over short periods of time that are high enough to cause mortality as evidenced in both incident data and in new study data.

EFED Response: Over the past 9 years, in the U.S., despite the widespread use of clothianidin, only a handful of U.S. incidents have been reported to EPA and, in those cases, government investigations could not definitively establish the role of clothianidin. While an unusual number of incidents associated with the planting of corn seed treated with neonicotinoid pesticides have been reported to EPA and Canadian authorities during the spring of 2012, the role of clothianidin in each of these incidents remains uncertain. EFED's initial assessment of these incident reports indicates that they involve relatively unusual conditions that are unlikely to occur repeatedly throughout the year or over widespread geographic areas. The majority of these recent incident reports indicate that there were dry windy conditions and bees were foraging in fields due to unusually warm conditions for the time of year. Another characteristic of the recent incident

reports was that while dead or dying bees were observed at the entrances to beehives, entire colonies were not lost.

So far as EPA is aware, an incident in Germany in 2008 and an incident in Slovenia in 2011 have been the only two large incidents (>1000 colonies affected) which have been definitively associated with clothianidin use. In both cases, the incidents resulted when fugitive dust, resulting from abrasion during the planting of clothianidin-treated maize seeds, was blown by unusual winds into adjacent fields which were in full bloom and where bees were actively foraging. In these and other reported incidents, there may also have been issues with regards to a failure to use proper sticking agents according to standard industry practices. According to reports of the German incident, which appears to be the most serious incident to date, some colonies showed only minor bee losses and only a slightly enhanced mortality, while other colonies showed severe damages. The scale of impact of the poisoning of colonies damaged varied between 10 – 90%. Total losses of colonies were reported in only a few cases. The relatively sporadic nature of the reported incident data in Europe and North America suggests that factors associated with such large scale acute mortality events are complex and not wide spread or common.

The new literature data the petitioners cite indicating regular exposures of bees to clothianidin residues include the Krupke et al. and Tapparo et al. studies discussed under point K. EFED cannot determine from these studies whether the nature of the exposures represented by these studies are a frequent or typical occurrence in the United States that could result in serious harm to bees. The available incident data indicate that these types of exposures to bees may occur when sowing is conducted during dry and windy conditions and bees are foraging on flowering plants that are adjacent to or near the fields where the treated seeds are planted. EPA is investigating whether currently available and future improvements in sticking agents, lubricants and venting of the sowing machines may further reduce the extent of potential exposures.

F. The petitioners allege that there may be synergistic effects between different pesticides and between the effect of pesticides in combination with pathogens and poor nutrition.

(i) Clothianidin in combination with other pesticides exhibits toxicity synergy:

EFED Response: Petitioners did not present data showing that nitroguanadine neonicotinoids in general, and clothianidin in particular, exhibit anything beyond additive toxicity[5] in combination with other pesticides. The petitioners cited only one study (Iwasa et al.) that presented data on synergism of mixtures. This study reported

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[5] Additive toxicity refers to the toxic effect on an organism when exposed to two pesticides together, equals the sum of their toxicity when that organism is exposed to each pesticide separately at the same doses. A synergistic interaction refers to the phenomenon when the toxic effect of the two pesticides together on the organism is greater than the sum of their separate toxic effects at the same doses. This is typically due to one of the pesticides inhibiting metabolism of the other.

synergism of cyano-substituted neonicotinoids (i.e., acetamiprid and thiacloprid) with two fungicides, triflumizole and propiconazole, but did not address clothianidin. Furthermore, the study did not find significant synergism between these fungicides and another nitroguanadine neonicotinoid insecticide, imidacloprid. EFED does not expect synergistic effects to occur between nitroguanidine neonicotinoids and fungicides because clothianidin and other compounds in this class are already resistant to degradation by the honey bee P450 enzymes while chemicals which inhibit these enzymes (such as piperonyl butoxide) may enhance the toxicity of pesticides like the cyano-substituted neonicotinoids which are more readily degraded by these enzymes. It was also unclear whether the levels of fungicides used in the study correspond to environmentally realistic doses. Finally the study authors found no corresponding synergism effect on honey bees when these pesticides were applied in the field at environmentally relevant rates.

The following section is EFED's analysis of the cited studies in support of the claim of synergism in pesticide combinations (Iwasa *et al*, Smodis *et al*, and Dai *et al*).
Iwasa, T., N. Motoyama, J.T. Ambrose, and R.M. Roe. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. 2004. Crop Protection. 23: 371—378.

The first citation, Iwasa *et al.*, (2004) claimed to show a synergistic effect of two fungicides with the neonicotinoids, acetamiprid (synergism ratio[6] of 244 with triflumizole) and thiacloprid (synergism ratio of 1141 with triflumizole). These two pesticides are in the cyano-substituted neonicotinoid subclass and show significantly less toxicity to honey bees than chemicals in the nitroguanadine-substituted sub-class; therefore, there is uncertainty in whether increased synergism will occur for nitroguanadine compounds including clothianidin, which already exhibit high toxicity to bees. Indeed, the Iwasa *et al.* study contradicts the petitioners' claims because the authors also investigated the effect with imidacloprid. In contrast to acetamiprid and thiacloprid, imidacloprid did not show a significant increase in toxicity (based on comparison of 95% confidence intervals) when used with piperonyl butoxide (PBO), triflumizole, or propiconazole with a maximum synergism ratio of 1.85 when used with triflumizole. Additionally, the study authors were unable to reproduce this synergistic effect in the field using acetamiprid and triflumizole in a tank mix at labeled application rates on alfalfa resulting in 4% mortality of caged honey bees after 24 hour exposure. Thus even when synergistic effects are possible on individual bees, it is highly uncertain that these occur in colonies under natural field conditions.

As Iwasa *et al.* implies, it is possible that synergistic effects are not seen between nitroguanidine neonicotinoids and fungicides because clothianidin and other compounds in the nitroguanidine sub-class are already resistant to degradation by the honey bee P450 enzymes while chemicals which inhibit these enzymes (such as PBO) may enhance the

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6 Synergism ratio was defined by the authors as the LD_{50} of the insecticide alone divided by the LD_{50} of the potential synergist and insecticide together.

toxicity of pesticides like the cyano-substituted neonicotinoids which are more readily degraded by these enzymes.

Since this study did not find synergistic effects with imidacloprid and synergistic effects that occurred in the laboratory between acetamiprid and triflumizole were not reproducible at the hive level in the field in this study, this study would likely be considered of low qualitative use for clothianidin risk assessment purposes.

Smodis, S.M., V. Kmecl, and A. Gregorc. 2010. Exposure to pesticides at sublethal level and their distribution within a honey bee (*Apis mellifera*) colony. Bulletin of Environmental Contamination and Toxicology. 85(2): 125-8.

The second cited study, Smodis *et al.* (2010), reported the presence of multiple pesticides, mostly acaricides used to control mites in the bee hives themselves, throughout the bee colony including coumaphos in royal jelly and fluvalinate in adult bees and bee larvae. The petitioners cite this as demonstrating that chemicals in the hive can be transmitted from bee to bee as well as to food and larvae, spreading throughout the colony. EPA has no evidence to confirm whether clothianidin (and other neonicotinoids) behaves similarly, and no information has been provided to indicate that clothianidin or other neonicotinoid residues accumulate in royal jelly. Further, EFED notes that coumaphos and fluvalinate are both applied directly inside the hive to control varroa mites; whereas, clothianidin and other neonicotinoids would never intentionally be applied directly to a hive. The relevance of this study with relation to synergism with clothianidin is unclear and the study would likely be considered of low qualitative use for clothianidin risk assessment purposes.

Dai P.L., Q. Wang, J-H. Sun, F. Liu, X. Wang, Y-Y. Wu, and T. Zhou. 2010. Effects of sub-lethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera lingustica. Environmental Toxicology and Chemistry. 29: 644—649.

The third cited study, Dai *et al.* (2010), examined sub-lethal effects of two pyrethroid insecticides (bifenthrin and deltamethrin) on honeybee health and found that these two chemicals significantly reduced colony fecundity due to reduced rates of egg laying and impaired ability of the colony to transition to a new queen, in addition to developmental effects on honeybee larvae. Because this study was conducted using two pesticides that are substantially different from clothianidin, EFED cannot determine the extent to which the results from this study would inform whether queens and larvae would be exposed to clothianidin residues and the extent to which exposure to clothianidin at environmentally relevant rates may reduce colony fecundity, transitioning to a new queen, or impair honeybee larvae. The relevance of this study with relation to synergism with clothianidin is also unclear, and the study would likely be considered of low qualitative use for clothianidin risk assessment purposes.

(ii) Synergy of pesticides and increased susceptibility to pathogens

EFED Response: The literature seems to indicate that there may be some synergy between exposure to many insecticides at sublethal levels and increased sensitivity to Nosema infestations. This appears to be true of a number of pesticides, including pesticides from neonicotinoid and other classes. The studies cited by the petitioners report this effect for imidacloprid, thiacloprid (a cyano-substituted neonicotinoid) and fipronil (a pyrazole). This may indicate that exposure to many different classes of insecticides, not just neonicotinoids, can contribute to increased sensitivity to pathogens. However, these studies do not show a dose-response effect, and the effects seem to be limited to individual bees, and do not extend to whole colonies. It is not known whether clothianidin would have a similar effect on the honey bee response to Nosema. Further, even if clothianidin has a similar effect, there remains uncertainty whether, at the levels of clothianidin residues typically encountered or the maximum expected concentrations in pollen and nectar, individual bees or the colony as a whole will experience a decreased immunity to Nosema infection similar to that seen in the literature. The levels used in the published studies are generally higher than the environmental levels of clothianidin to which honey bees would likely be exposed. EFED notes, however, that certain use patterns, which are not as widespread as clothianidin seed treatments on corn, such as some foliar applications, may result in residues in pollen and nectar approaching these concentrations. Where residue analysis has been conducted, colonies which were eventually determined to have succumbed to CCD had increased occurrence and growth of pathogens, including Nosema infections, but were not found to contain elevated levels of any neonicotinoids including clothianidin (vanEngelsdorp et al, 2009).

The following section is EFED's analysis of the cited studies in support of the claim that neonicotinoid pesticides act to reduce honey bee tolerance of pathogens such as Nosema (Pettis et al., Vidau et al., Alaux et al., and Wu et al).

Pettis J.S., vanEngelsdorp D, Johnson J, Dively G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwissenscaften. 99: 153—158

Pettis *et al.* 2012 reported increased pathogen growth among individual bees (fed Nosema contaminated sucrose solution) reared in colonies exposed to imidacloprid at 5 and 20 ppb in spiked protein patties. However, no dose-response was observed (*i.e.* the increase in spore load was constant regardless of the exposure concentration). Additionally, the authors reported that newly emerged bees from the 20 ppb spiked colony (with imidacloprid below the LOD of 0.1 ppb) were significantly lighter in weight in one trial, but showed no difference in a second trial. Newly emerged bees from the 5 ppb spiked colony did not show any weight differences compared to control bees. Further, contamination in the control bee bread also may confound the study results. Interestingly, though the article reported that individual bees exposed to imidacloprid showed an increase in *Nosema* spore production in the laboratory, the exposed parent colonies were reported not to show increased *Nosema* spore production. Throughout the course of the study, 8 of the 30 colonies used tested positive for *Nosema* (3 control, three 5 ppb a.i. and two 20 ppb a.i.). In fact, these infestations were reported to be highest in

the control and 5 ppb colonies and lowest in the 20 ppb colony (average spore counts of 4.3, 2.9, and 0.5 million spores per bee, respectively). Therefore, the biological relevancy of this study to bee colonies under natural field conditions is highly uncertain. This study would likely be considered of medium qualitative use for clothianidin risk assessment purposes.

Vidau, C., M. Diogon, J. Aufauvre, R. Fontbonne, B. Vigues, J.L. Brunet, C. Texier, D.G. Biron, N. Blot, H. El Alaoui, L.P. Belzunces, F. Delbac. 2011. Exposure to Sublethal Doses of Fipronil and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by *Nosema ceranae*. PLoS ONE 6(6): e21550.

Vidau *et al.* reported that exposure to fipronil and thiacloprid had no effect on the mortality of uninfected individual honeybees compared to control bees, however exposure to the two pesticides did increase the rate and overall mortality to honeybees infected with *Nosema.* Additionally, the authors reported that uninfected honey bees did not display any signs of intoxication after being exposed to these two pesticides, but that at the tested levels of exposure, infected bees experienced aggressiveness and tremors in the first days of exposure and ataxia in later days. However, spore production in infected bees was reduced in those exposed to fipronil and increased in those exposed to thiacloprid. EFED notes that these two insecticides are significantly different (one a cyano-substituted neonicotinoid and one a pyrazole) than clothianidin, both in their chemical structure and level of lethal toxicity to honey bees. Therefore, EFED thinks that these results do not demonstrate that bees exposed to clothianidin would display similar susceptibility to *Nosema* or other pathogen infection at sublethal levels. As with the Pettis et al. 2012 study, the actual relevancy of this study to bee colonies under natural field conditions is highly uncertain and given that it was not conducted on a nitroguanidine neonicotinoid it would likely be considered of low qualitative use for clothianidin risk assessment purposes.

Alaux, C., J.L. Brunet, C. Dussaubat, F. Mondet, S. Tchamitchan, M. Cousin, J. Brillard, A. Baldy, L.P. Belzunces and Y. Le Conte. 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology. 12(3): 774—782.

Similar to Pettis *et al.*, the Alaux *et al.* study (MRID 48077922) reported that after exposure to imidacloprid, mortality in bees infected with *Nosema* was higher than in control bees or in bees only exposed to imidacloprid or in bees infected with *Nosema* with no imidacloprid exposure. The authors indicate that exposure to imidacloprid and infection with *Nosema* generally appeared to have an additive effect on the mortality rate for the 0.7 and 7 ppb treatment groups, but that there was a synergistic effect at the highest concentration (70 ppb). They also observed that *Nosema* infected bees consumed significantly more sucrose than control bees or bees only exposed to imidacloprid and that infected bees who were exposed to imidacloprid consumed the most sucrose. Some control bees may have accidentally been infected with *Nosema* at the beginning of the experiment (but at levels significantly below the treatment groups). Interestingly, bees exposed to imidacloprid had a slightly lower number of *Nosema* spores than bees not

exposed to imidacloprid both non-infected bees compared to control bees and infected bees with imidacloprid treatment compared to infected bees alone.

The authors also examined imidacloprid and *Nosema* effects on bees' immune function. Phenoloxidase enzymatic activity and total haemocyte count, measures of individual immunity, were not affected by *Nosema* or imidacloprid exposure. *Nosema* infection and exposure to imidacloprid together reportedly decreased glucose oxidase (GOX)-specific activity, which according to the study authors is considered a measure of social immunity; however the authors did not indicate at what concentration of imidacloprid the GOX-specific activity was tested. The authors performed these experiments using dimethyl sulfoxide (DMSO) as a solvent for imidacloprid. The chemical properties of DMSO are known to increase the permeability of biological membranes, and it is unclear how the use of this solvent may have affected absorption, distribution, excretion and metabolism of the test compound. EFED typically considers studies that use this solvent as having low qualitative utility in risk assessments.

Additionally, the reported concentration of imidacloprid at which synergistic effects occurred (70 ppb) is far above the typical expected concentrations of either imidacloprid or clothianidin in pollen and nectar, though certain use patterns may result in residues approaching these levels. Given that the study used DMSO, the lack of effects at environmentally relevant concentrations, the contradictory information of decreased spore production but increased toxicity with imidacloprid, *Nosema* contaminated controls, and the lack of information regarding the imidacloprid concentrations used in the GOX experiment, this study appears to be of low qualitative value for use in the clothianidin risk assessment.

Wu, J.Y., C.M. Anelli, and W.S. Sheppard. 2011. Sub-lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity. PLoS ONE 6(2): e14720.

Wu et al. examined sub-lethal effects of residues of fluvalinate and coumaphos in brood comb on worker honey bees. As noted previously, coumaphos and fluvalinate are both applied directly inside the hive to control varroa mites while clothianidin and other neonicotinoids would never intentionally be applied directly to a hive. This study was not conducted on neonicotinoids and appeared to have contamination in controls over the course of the study, and so its scientific validity under any circumstances is questionable. At best, it appears to be of low qualitative value for use in the clothianidin risk assessment.

G. Petitioners allege that the first CCD losses in U.S. correlate with increasing neonicotinoid pesticide use, indicating causality.

EFED Response: Petitioners did not provide data that shows that the incidence of CCD in the U.S. is correlated with the use of pesticides in general or with the use of neonicotinoids in particular. The petitioners cite VanEngelsdorp et al., 2010 analysis of factors associated with CCD and point out that they found that pesticides accounted for 6

of the 19 variables having the greatest discriminatory power to predict CCD occurrence. However, none of these six pesticide related variables were linked specifically to either the class of neonicotinoid pesticides or a particular neonicotinoid pesticide. Additionally pesticide residue analyses from national surveys of commercial honey bee colonies indicate that neonicotinoids in general and especially nitroguanidine neonicotinoids and clothianidin in particular are detected relatively infrequently in migratory colonies (Mullin et al., 2010) and that where residue analysis was conducted, colonies which were eventually determined to have succumbed to CCD did not contain elevated levels of neonicotinoids including clothianidin (vanEngelsdorp et al, 2009). While surveys of stationary colonies have resulted in more frequent detections of neonicotinoids and at higher levels than those reported for migratory colonies, these surveys were associated with colonies primarily in residential settings and these colonies did not exhibit CCD (unpublished data. Personal Communication, Stoner 2010).

H. Petitioners allege that neonicotinoid pesticides are known to interfere with bees cognition and orientation and that sub-lethal exposures are cumulative and affect foraging behavior, mobility and communication of social insects, shifts in hive roles, and learning impairments

EFED Response: From the petitioners' cited studies (as well as other available data), there appears to be evidence that imidacloprid can have sub-lethal inhibitory effects on honey bees including effects on mobility, feeding activity and memory and associative learning capabilities. It is unclear whether these effects are permanent or transitory. EFED cannot determine from the cited studies whether these effects would occur in the field and if so, whether the degree to which they occur would affect populations of honey bees. The minimum concentrations at which significant biological effects occurred in the majority of the studies presented here and in the literature overall (Medrzycki et al.—100 ppb,Decourtye et al.—approximately 94 ppb, Yang et al.—50 ppb) are not typically present in the field or in chronic concentrations present in nectar and pollen from the most widespread use patterns of clothianidin (though Colin et al. found an inhibitory effect on bees feeding on sucrose solution at 6 ppb). No studies were cited to defend the petitioners' assertion of shifts in hive roles. EFED was unable to determine from the cited studies whether clothianidin would act in the same manner as imidacloprid because there is a lack of data to show that clothianidin's chronic mode of action is completely comparable to imidacloprid.

The following section is EFED's analysis of the cited studies in support of the claim that neonicotinoid pesticides cause sublethal effects such as interfering with bee cognition and orientation affecting foraging behavior, mobility and communication and shifts in hive roles and learning impairments (Medrzycki et al., Colin et al., Decourtye et al., and Yang et al.).

Medrzyck P, Montanari R, Bortolotti L, Sabatini AG, Maini S, and C Porrini. 2003. Effects of imidacloprid administered in sub-lethal doses on honeybee behavior. Laboratory test. Bulletin of Insectology. 56(1): pp 59-62. MRID 47800529

The Medrzycki *et al.* study reported decreased mobility of bees exposed to sucrose solution containing 100 and 500 ppb imidacloprid. The tests were conducted with both a single application and ad libitum feeding of contaminated solutions. However, this effect was transitory, beginning 30-60 minutes after application and disappearing after 2 hours. The authors also reported that control bees were usually all stationary or all running compared to treated bees, where each single bee did not seem to be influenced by the behavior of other bees. The authors support this by reporting that the standard deviation of the number of running honey bees was significantly higher in the treated groups compared to untreated bees, indicating that treated bees acted in a more random manner. There did not appear to be a dose-response relationship from this effect with the standard deviation in the 500 ppb treatment level appearing to be closer (though still significantly different) to the control group than the 100 ppb treatment level. From this difference in control and treatment standard deviations, the authors conclude that treated bees experienced decreased communicative capacity.

It is likely that, at the relatively high exposure levels examined in the study, bees would exhibit responses that are consistent with the acute toxicity of the compound and not the sub-lethal and transitory reactions that this experiment describes. However, the extent of the exposure in the study is unclear since the amount of active ingredient actually consumed by treated bees is not provided. It is also unclear that these high concentrations would occur with any frequency in bee-collected pollen and nectar from imidacloprid seed-treated plants as no data are available showing imidacloprid or other nitroguanidine neonicotinoids present in those concentrations in pollen or nectar for a sustained period of time (although contamination from dust from abraded seed could result in these concentrations for a day or two, but likely not longer) from imidacloprid or clothianidin seed treatments. There is also a large degree of uncertainty in using a measure of dispersion (in this case, standard deviation) and without a clear dose-response relationship to infer a broad biological effect of diminished communicative capacity. The results of the study indicating diminished mobility at high concentrations of imidacloprid would likely be considered of medium utility for clothianidin risk assessment purposes, but the conclusion of decreased communicative capacity has not been adequately tested and would likely be considered insufficient for clothianidin risk assessment purposes.

Colin ME, Bonmatin JM, Moineau I, Gaimon C, Brun S, and JP Vermandere. 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Contam. Toxicol. 47: 387-395. MRID 47523408

Colin *et al.* in a semi-field study (with 2300 bees/colony) reported no decrease in bee attendance at a feeder in the presence of 6 µg/kg imidacloprid, but that activity (defined as feeding on sucrose solution) at this concentration was decreased compared to controls during four days. Bees exposed to fipronil (2 ug/kg) were reported to exhibit both a decrease in attendance and a decrease in activity. The study used an unbalanced design with 8 controls temporally spaced prior to the experiment and one control and three treatment colonies used during the treatment. The authors then pooled the data from the 9 control plots. EFED typically recommends using only concurrently run control and

treatment groups and an equal number of control and treatment groups. Of the three treatment groups, two exhibited a significant decrease in bee activity by the 4th treatment day, while one group experienced no decrease. The authors did not quantify this difference in the article other than to report that it was significant. The study authors did not report food (sucrose) consumption in the feeder nor any colony parameters making the relevancy of this study to effects on full colonies under natural field conditions somewhat unclear, but the authors did use environmentally relevant concentrations of imidacloprid. This study would likely be considered of low qualitative use for clothianidin risk assessment purposes because of its unbalanced test design with only one control hive, pooling of controls with previous data prior to conducting statistical analysis, inability to define a dose-response given that only one treatment concentration was tested, unclear definition of "active" bees and how this parameter was observed and validated, and lack of measurement of food consumption by the bees.

Decourtye A, Armengaud C, Renou M, Devillers J, Cluzeau S, Gauthier M, and MH Pham-Delègue. 2004. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.) Pesticide Biochemistry and Physiology. 78: 83-92. MRID 47523405

Decourtye *et al.* studied the proboscis extension reflex (PER) response in honey bees after exposure to spiked sucrose solutions with imidacloprid at 12 ng/bee (approximately 94 ppb based on a honey bee weight of 0.128 g) and 0.12 ng/bee (approximately 0.94 ppb). The authors conducted three tests: a study on the acquisition process (conditioned response), one on retrieval performance and finally one on imidacloprid's effect on short-, medium-, and long-term retention of conditioning. In the first test, the study authors reported that 30 minutes after treatment at the higher rate, bees showed significantly lower conditioned responses than untreated bees. In the second test, there was a significant decrease in the percentage of bees retaining the conditioned response at 1 hour and 24 hours after treatment at the 12 ng a.i./bee rate. In the third test, the authors reported that after conditioning, imidacloprid had no effect on short-term (imidacloprid presented at 30 seconds to 15 minutes after conditioning) or long-term (imidacloprid presented at 24 hours after conditioning) retention, but did significantly impair medium-term retention (imidacloprid presented at 15 minutes and 1 hour after conditioning). The minimum exposure level that resulted in effects to bees (12 ng/bee or approximately 94 ppb) is unlikely to be frequently encountered in pollen and nectar under the most widespread clothianidin use patterns.

The study authors also did a cytochrome oxidase (CO) histochemistry study using six bees per treatment and found an increase in CO staining observed in lip and basal rings of the mushroom bodies (MB) calyces 30 minutes after imidacloprid treatment. The petitioners cite this as evidence of imidacloprid acting detrimentally on parts of the bee brain involved with associative and contextual memory. The Agency does not typically make use of *in vitro* or suborganismal data since its links to apical endpoints is highly uncertain, therefore the PER data would likely be considered of medium qualitative use, but the suborganismal data would likely be considered of low qualitative use for clothianidin risk assessment purposes. As with many sublethal effects measured on

individual bees, there is considerable uncertainty how these measurement endpoints relate to apical effects on individual bee and on whole colony survival, growth and reproduction.

Yang EC, Chuang YC, Chen YL, and LH Chang. 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). Journal of Economic Entomology. 101(6): 1743-1748.

Yang *et al.*, (MRID 47800532) examined foraging behavior for marked honey bees returning to artificial feeders with 50% sugar water. A bee's normal visiting time to return to an artificial feeder, based on the control group was reported to be <300 sec (with an average of 150 sec). Imidacloprid was prepared in 12 solutions of sugar water between 40 and 6,000 µg/L and dissolved in DMSO (after the authors reported that using acetone as a solvent had a negative effect on the number of abnormal bees). When replacing the control artificial feeder with the treatment artificial feeders (the experiment did not use concurrent controls and treatment groups), imidacloprid doses ≥ 50 µg/L were reported to result in significantly increased number of bees taking longer than 300 sec to return to the feeder. The percent of bees exhibiting longer foraging times increased with increasing dose of the pesticide. The study authors reported that at concentrations greater than 1200 µg/L, all bees showed abnormal foraging behavior. However, all of the bees exposed to concentrations lower than 1600 µg/L were able to recover the next day.

Residues of imidacloprid and metabolites were not analyzed in the sucrose solutions or bees and it is not possible to know the exact amount of a.i. in the treated bees at any specific time. Additionally, the study does not provide the information necessary to determine if bees could have foraged in other areas; therefore the actual exposure levels are unknown.

The authors performed these experiments using dimethyl sulfoxide (DMSO) as a solvent for imidacloprid. The chemical properties of DMSO are known to increase the permeability of biological membranes, and the use of this solvent may have affected absorption, distribution, excretion and metabolism of the test compound. EFED typically considers studies which rely on DMSO to have low utility in risk assessments. Additionally, the reported minimum concentration at which effects occurred (50 µg/L) is above the typical expected concentrations of either imidacloprid or clothianidin in pollen and nectar, though certain use patterns, such as some foliar applications, may result in residues approaching these levels. Given the lack of concurrent controls, use of DMSO and the relatively high concentrations that were tested, this study would likely be considered of low qualitative use for clothianidin risk assessment purposes.

I. The petitioners allege that bees are regularly exposed to sublethal levels of clothianidin through contaminated pollen, nectar and guttation water can suffer chronic effects.

EFED Response: The petitioner cites Krupke et al. as evidence that clothianidin concentrations in corn pollen are ten times higher than reported from experiments on

clothianidin seed treated canola. However, Krupke et al. actually found clothianidin concentrations of 3.9 ppb in pollen gathered from plants grown from treated maize seed, which is similar to the levels reported in pollen from seed treated canola (Cutler and Dupree, 2007, as well as registrant submitted studies, MRIDs 45422431, 45422432, 45422433, 45422435, 45422436, 45422437). Krupke et al did find higher levels (up to 88 ppb) in pollen samples from returning foragers, however the relationship between these concentrations in pollen and planting was highly variable (between the LOD and 88 ppb in hives in treated areas and between the LOD and 20.1 ppb in hives in untreated areas) with only two hives in both treated and untreated areas and these concentrations appeared to decrease over time. Further, these reported concentrations may have been a function of contaminated dust after corn planting rather than systemic expression in pollen and nectar.

The petitioner also includes data on imidacloprid concentrations in corn pollen (Bonmatin et al, 2003, 2005b). Bonmatin (2003) reported imidacloprid concentrations in a very small sample (n=5) of maize pollen of 1-3 ppb with a mean of 2 ppb. Bonmatin et al. (2005b) reported imidacloprid concentrations in maize pollen collected from the plant of 2.1 ppb (n = 47) and 0.6 ppb (n = 11) in pollen collected at hive entrances.

The petitioner also cites Greatti et al, (2006) who reported contaminated dust from imidacloprid treated corn seed on flowers (predominately dandelions) reaching concentrations up to 123.7 ppb and declining to 4.9 ppb four days after sowing. This contamination was clearly a result of the sowing operation and not an effect of systemic translocation of imidacloprid from the seed into pollen, and the study author's results do not indicate that these high concentrations last for extended periods of time.

Petitioners did not provide any data that indicate that honey bees exposed to the concentrations of clothianidin and imidacloprid detected in pollen collected from maize anthers will exhibit the types of sub-lethal symptoms that the petitioners argue are equivalent to those experienced by hives affected by CCD such as effects on mobility, feeding activity and memory and associative learning capabilities. Other use patterns may result in higher clothianidin concentrations in pollen and nectar, but these use patterns are not as widespread and common as the seed treatment on corn use pattern.

No survey or incident data is available to indicate a connection between CCD and neonicotinoid pesticides. Pesticide residue analyses from national surveys of commercial honey bee colonies (vanEngelsdorp et al, 2009) indicate that neonicotinoids are detected relatively infrequently in migratory colonies and that colonies which were eventually determined to have succumbed to CCD did not contain elevated levels of neonicotinoids including clothianidin. While surveys of stationary colonies have resulted in more frequent detections of neonicotinoids and at higher levels than those reported for migratory colonies, these surveys were associated with colonies primarily in residential settings and these colonies did not exhibit CCD.

From the petitioners' cited studies one study, Girolami et al., appears to provide data to support the presence of clothianidin and other neonicotinoid insecticides in guttation

water on seed treated plants. These exposures can be at levels toxic to bees immediately after seedling emergence and are still at detectable levels up to three weeks following emergence. The study authors did not indicate the rate at which these levels declined and the degree to which bees are present when contaminated guttation water is available or the degree to which bees will make use of guttation water or supply it to the hive is unknown.

The following section is EFED's analysis of the cited studies in support of the claim that bees regularly exposed to sublethal levels of neonicotinoid pesticides through contaminated pollen and nectar and guttation water can suffer from chronic effects (Girolami *et al.*, 2009 and Greatti *et al.*, 2006. Bonmatin *et al.* 2005b).

Girolami V, Mazzon L, Squartini A, Mori N, and A. Marzaro. 2009 Translocation of Neonicotinoid Insecticides from Coated Seeds to Seedling Guttation Drops: A Novel Way of Intoxication for Bees. Journal of Economic Entomology, 102(5): 1808—1815.

Girolami *et al.* conducted experiments with corn seed treated with imidacloprid (0.5 mg ai/seed), clothianidin (1.25 mg ai/seed) and thiamethoxam (1.0 mg ai/seed) and measured concentrations of these a.i.s in guttation water for the first three weeks following seedling emergence. The study authors also investigated the effect of guttation water from seed-treated corn on honey bees in the lab. They reported finding the presence of neonicotinoids in all samples during the three-week study period and reported mean concentrations of 47 ± 9.96 mg/L, 23.3 ± 4.2 mg/L, and 11.9 ± 3.32 mg/L for imidacloprid, clothianidin and thiamethoxam respectively. Peak concentrations above 200 mg/L for imidacloprid and 100 mg/L for clothianidin and thiamethoxam were also reported. It was unclear whether these mean concentrations were from initial sampling after seedling emergence or for the entire three-week study period. Despite the lower amount of active ingredient per seed for imidacloprid, mean and peak concentrations of imidacloprid in guttation water were significantly higher than in guttation water from clothianidin and thiamethoxam treated seed. The honey bees offered guttation water from clothianidin and thiamethoxam seed treated corn were reported to experience earlier irreversible wing block (paralysis of the thorax muscle which controls wing movement) and mortality at lower concentrations than for bees drinking guttation water from imidacloprid treated plants. The authors did not report whether concentrations in guttation water decreased over the three week period. This exposure data of the different active ingredients in guttation water would likely be considered of high qualitative use for clothianidin risk assessment purposes; however, the study does not address whether the bees would have consumed the guttation water used in the study had the authors not added sucrose to increase its attractiveness to the bees.

Greatti M, Barbatttini R, Stravisi A, Sabatini A, and S Rossi. 2006. Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho dressed seeds. Bulletin of Insectology. 59(2): 99-103.

Greatti *et al.*, looked at imidacloprid released as part of the sowing/drilling procedure on nearby vegetation and they also examined the effect of an unidentified adjuvant (sticking

agent). They examined imidacloprid residues on paper filters placed by the fan of the seed driller and samples of grass and dandelion flower heads. Five trials were conducted, two with and without the corn seed treatment and 1 with both the seed treatment and the adjuvant. They reported increasing imidacloprid on the paper filters with exposure time. The authors reported that the adjuvant reduced, but did not eliminate this release and that contamination of succeeding trials occurred despite washing the seeding equipment between trials; however, the authors did not report what types of sticking agents and/or lubricants were used in the study. The authors reported dispersion of imidacloprid in grass and flower samples collected in the borders of each field in each trial where imidacloprid was used. Residues on flowers in one treatment were up to 123.7 ng/g (ppb) and persisted for four days while the other two treatments were 25.1 and 22.4 ng/g. These latter two treatments were exposed to rain that may have washed away the residues on days following sowing. Residues on grass were lower than residues on flowers. The data on imidacloprid residues on grass and flowers would likely be considered of low qualitative use for clothianidin risk assessment purposes since residues were on entire flowers and plant parts and not specifically measured on pollen or nectar.

Bonmatin JM, Marchand PA, Carvet R, Moineau I, Bengsch ER, and Colin ME. 2005. Quantification of imidacloprid uptake in maize crops. Journal of Agricultural Food Chemistry. 53: 5336-5341

Bonmatin et al. 2005b collected maize plants and pollen and reported that pollen collected from organically farmed areas or areas that had not been treated with imidacloprid in the last three years (n = 3) had no imidacloprid detected. In samples from imidacloprid seed treated areas, they reported average levels of 6.6 µg/kg (n = 17, range <LOD -- >10 µg/kg) for stem and leaves, 4.2 µg/kg (n=48, range LOD—33.6 µg/kg)for panicles, 2.1 µg/kg for plant collected pollen (n = 47, range <LOD—18 µg/kg) , and 0.6 µg/kg (n = 11, range <LOD—10 µg/kg) for pollen collected at hive traps. This information on imidacloprid residues in corn pollen would likely be considered of medium qualitative use for clothianidin risk assessment purposes.

J. Petitioners claim that neonicotinoid pesticide use causes disruption of a bee's microbial community (fungi, bacteria, viruses)

EFED Response: The study cited by the petitioners does not show a negative effect of neonicotinoid pesticides in general, and clothianidin in particular, on the enteric microbial community of the honey bee.

The following section is EFED's analysis of the cited study in support of the claim that there is an adverse effect of neonicotinoid pesticides on the enteric microbial community of bees. The petitioners cite Anderson *et al.* (2011) on the effects of pesticides on the role of microbiota in bee colony health.

Anderson KE, Sheehan TH, Eckholm BJ, Mott BM, and G DeGrandi-Hoffman. 2011. An emerging paradigm of colony health: microbial balance of the honey bee and hive (*Apis mellifera*). Insect. Soc. 58: 431-444.

This article is a literature review of some of the published information on the microbial community relation to the honey bee (in an individual bee's digestive system, bee products, and overall in the hive). No information is presented in the review regarding the effect of neonicotinoid insecticides on the microbial community and this study is considered of low qualitative use for clothianidin risk assessment purposes.

K. The petitioners allege that critical new studies demonstrate high acute and chronic residue levels and that available modifications to planting technology (seeds and sowing equipment) have limited effects. Therefore, exposure to bees will occur regardless of any label mitigations.

EFED Response: The Krupke et al. study does provide information regarding high concentrations of clothianidin in talc waste remaining in seed planters after planting treated seed, persistence of residues in soil after more than one growing season, and concentrations in corn pollen and on dandelion flowers on which bees may be foraging. Generally, the high concentrations in talc waste would be expected to kill any bees that contact it. The petitioner cited that levels of contamination in bee-collected pollen at the time of planting were 10x higher than reported from an experiment on clothianidin-treated canola seed (Cutler and Dupree, 2007). However, there was considerable amount of variation in clothianidin residues in pollen between treated and untreated sites with one of the two colonies from a treated site showing no detectable residues while both of the hives from the untreated sites had clothianidin residues. The source of the contaminated pollen is unclear since the corn would not be shedding pollen when it was planted and it is also unclear whether honey bees would typically be exposed to these concentrations in pollen and nectar. EFED cannot determine from the study whether the average levels of clothianidin residues that the study authors report in pollen and nectar would cause sub-lethal effects similar to CCD symptoms in honey bees, though the maximum residues reported would be more likely to cause sub-lethal or even lethal effects.

The Tapparo et al. study indicates that under certain conditions, emissions of clothianidin-contaminated dust following pneumatic drilling can occur, even when specific modifications to the drilling machines are made. The modifications tested however, did appear to result in a decrease in the extent to which clothianidin drifted from the machine. Improvements in seed coatings between 2009 and 2010 also appeared to decrease, but not eliminate, emissions of dust containing clothianidin residues. The study did not include information as to whether sticking agents were used in the seed treatments or whether additional "lubricants" such as talc or graphite were used to aid in moving seed through the sowing equipment. The study was conducted with European sowing machines, and it is unclear if there are differences in venting between those and equipment used for seeding in the U.S. The free foraging bees used in this study were conditioned to fly in close proximity to seeding equipment and EFED was unable to determine whether bees would naturally be attracted to a field that is being seeded and the extent to which they would typically encounter exhaust from seeding equipment.

EPA cannot determine from these studies whether the nature of the exposures represented by these studies are a frequent or typical occurrence in the United States that could result in serious harm to bee colonies. However, the relatively sporadic nature of the incident data suggests that factors associated with such high exposures are complex and not wide spread or common. The available data indicate that these exposures to bees may occur when sowing is conducted during dry and windy conditions and bees are foraging on flowering plants that are adjacent to or near treated fields. Currently available and future improvements in sticking agents, lubricants and venting of seeding equipment may further reduce the extent of potential exposures.

The following section presents EFED's analysis of the Krupke *et al.* and Tapparo *et al.* studies relative to the petitioners' claim that these studies provide evidence of unacceptable adverse effects to honey bees posed by clothianidin through exposure pathways not previously considered by the Agency. Specifically the studies identify residues in soil (present even after untreated for two years), in pollen (hive collected and fresh and at levels 10x the reported canola levels in the Cutler and Dupree 2007 article), on dandelion flowers and other non-target plants, and residues in waste talc.

Krupke, C. H., G. J. Hunt, B. D. Eitzer, G. Andino, K. Given. 2012. Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields. PLoSOne 7(1): e29268.

Krupke *et al.*, examined pesticide (several, but mainly clothianidin) residues in waste talc, soils (both currently treated and previously treated fields), pollen (from maize anthers, collected from foraging bees and inside hives), live and dead bees from within or near hives and nectar collected from hives. The authors reported clothianidin residues in waste talc from treated seed of 15,000 ppm a.i. clothianidin (untreated maize seed had contamination and resulted in waste talc concentrations of 12 ppm a.i.). Soils surrounding the study area that were in maize and/or soy production were reported to contain 2.1-9.3 ppb a.i. Soils in unplanted fields near an apiary, which had been previously reported to have a minor bee kill, contained 6.0—8.9 ppb a.i. clothianidin as well as significant residues of metolachlor and atrazine. The authors reported that even where no treated seed had been planted for two preceding growing seasons, samples continued to contain clothianidin. Dandelions from these unplanted fields were reported to contain 1.1—9.4 ppb clothianidin.

Pollen collected from anthers of seed treated corn was reported to contain 3.9 ppb a.i. (pollen from untreated corn plants contained 0.3 ppb). They reported conflicting data for pollen sampled from returning foragers with pollen sampled from control hive foragers containing similar or higher clothianidin residues (1.1—20.1 ppb) prior to planting than after planting (<1.0—6.7 ppb). The hives near seed-treated corn were reported to exhibit a similar pattern with clothianidin residues decreasing from 88 ppb two days prior to planting to 3.9 ppb post planting in pollen collected from foragers returning to one hive and no residues detected from pollen collected from foragers returning to a second hive. They hypothesized that this may reflect the high variability in the types of pollen being brought back to the hive, but present no evidence quantifying this variability. This study,

however, does not address the uncertainty as to whether the elevated clothianidin residues in pollen are due to contamination of local pollen by drift of dust from abraded seed coatings during planting or whether the residues in pollen were due to translocation of systemic residues.

Dead and dying bees in front of affected hives were reported to contain 3.8—13.3 ppb a.i. clothianidin, while bees which appeared normal had clothianidin residues < 1.0 ppb. Clothianidin was not detected in nectar from either apparently normal of affected hives, but pollen from a normal hive contained 2.9 ± 1.3 ppb clothianidin and 6.2 ± 4.9 ppb thiamethoxam while pollen from an affected hive contained 10.7 ± 2.3 ppb clothianidin and 20.4 ± 2.7 ppb thiamethoxam. However, due to the low sample size (n = 2), it is unclear how significant these differences are. Indeed, throughout this article the sample size for most residues measured is unclear and may be small, possibly affecting the reliability of the results (though n = 100 for the pollen from corn anthers samples, but n = 7-10 for dandelions and the sample size for other measurements was not reported). Additionally, meteorological conditions at the time of sowing that may have facilitated the movement of dust were not reported.

There are a number of other uncertainties and limitations associated with this study. The study authors report that corn pollen was sampled directly from tassels; however, it is uncertain when this corn was planted relative to the time when the crop would be typically sown in Indiana. The study authors also report that maize pollen represented from 2.6 to 82.7% of the pollen when maize was planted; however, the source of this pollen is not stated. EFED believes the source of pollen was different given that maize would not be shedding pollen at the time of year when it is typically planted. Previous exposure history of the field used for sampling soil was also not provided. The study does not provide information as to the extent to which the talc sampled from the seeding equipment manifold is released during seeding. Although the highest residues reported in the study were associated with talc (residues in the parts per thousand), it is unclear whether this talc would be readily distributed to the surrounding environment through the seeding process. Talc from untreated seed was found to contain detectable residues which the authors attributed to cross contamination, however there is uncertainty how waste talc from untreated seed may also have been contaminated.

Also, there is a considerable amount of variation in reported clothianidin residues in pollen between treated and untreated sites in the study. Colony #6 from a treated site showed no detectable residues of clothianidin while hive #5 from a untreated site had residues at 20.1 ppb; hive #7 from an untreated site showed frequent clothianidin detections pre- and post-planting with residues ranging between <LOD to 13.1 ppb. The limited and contaminated control data makes it difficult to confirm the extent to which residues detected in the study colonies could be directly related to dust from abraded seed coatings. Although plants growing in the vicinity of affected colonies exhibited residues of clothianidin, the source of these residues is unknown as again, the prior exposure history of the collection area is not provided. Also, the distance that the plants were collected from sites recently sown with neonicotinoid-treated fields is not stated.

The authors acknowledge that bees may forage over relatively large distances and they did not presume that bees would have foraged exclusively in the areas adjacent to the sampling sites; a more comprehensive understanding of other crops in the treatment area would be helpful. Although the researchers hypothesize that neonicotinoid residues in plants may have been a result of uptake and/or dust from treated soils, contamination from dust-off may also be a source.

The exposure data on clothianidin concentrations in waste talc, soil and corn pollen would likely be considered of medium qualitative use for clothianidin risk assessment purposes, but the exposure data in hive collected pollen, given the low sample size and presence of clothianidin in hives in untreated areas, would likely be considered of low qualitative use for clothianidin risk assessment purposes.

Tapparo, a., D. Marton, C. Giorio, A. Zanella, L. Soldà, M. Marzaor, L. Vivan, and V. Girolami. 2012. Assessment of the Environmental Exposure to Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds. Environmental Science and Technology 46: 2592 – 2599.

The petitioners cite the Tapparo *et al.* study as evidence that atmospheric emissions of particulate matter by standard seed drilling machines result in high exposure levels for bees and colony loss phenomena and that differing planter exhaust configurations had limited effect on emissions.

Tapparo *et al.* examined particulate and atmospheric emission of neonicotinoid concentrations following corn sowing with several models of pneumatic drilling machines and the effect on honey bees. The seed coatings used included 2008, 2009 and 2010 clothianidin seed, 2010 thiamethoxam seed and 2010 fipronil seed. The machines used included a Ribouleau Monosem NG plus (both unmodified and modified with a double pipe to funnel air towards the soil) and a Gaspardo model with a deflector at the fan outlet to direct the air stream towards the soil. They measured particulate matter, total suspended particulate (TSP) and PM_{10} (particles $\leq 10\mu m$). They also measured residues in dead bees found in the field or close to the beehive and kept bees in cages at varying distances next to the drilling machines and under differing humidity conditions.

The study authors reported that 1 hour of drilling activity can generate 280 $\mu g/m^2$ active ingredient clothianidin in dry deposition with 2008 seed (2009 seed contained approximately 50% of this value) and concentrations in total suspended particulate (TSP) at the field margin were 0.24 and 0.10 $\mu g/m^3$ for 2008 and 2009 seed coatings respectively. The 2010 clothianidin seed coating was reported to emit more particles, but they had a larger diameter reducing their capability to be wind dispersed. Approximately 0.5% of 2008 and 2009 clothianidin was released into the atmosphere as coarse particles (~0.45 g/ha), while 2010 clothianidin seed had a higher emission factor (1.84% or 1.53 g/ha), but these particles were larger (0.5—2 mm) that deposit quickly. The modified Monosem planter was reported to reduce clothianidin concentrations in TSP by 2.5—7x at 5 m from drilling machine and 3.5—11x at 10m (2009—2010 data), while the Gaspardo machine (2010 seed only) reduced clothianidin concentrations in TSP by

approximately 3x compared to the unmodified Monosem at 5 m and 6.6x at 10 m. The 2010 coated seed was reported to have a reduction of ~ 2x for the unmodified Monosem at 5 m and 2.5x at 10 m, while the modified Monosem had similar clothianidin emissions at both 5 m and 10 m from the seeder for the two years seed coatings. Regardless of the modifications, significant amounts of micrometric particles containing clothianidin were reported to be emitted. Meteorological conditions at the time of sowing were not reported and it is unclear whether climatic conditions may have facilitated the movement of dust.

The study authors observed the death of a significant, but unreported number of bees whose beehives were ca. 100 m from the sowing field. It was not reported whether any colonies were lost. Even when using the Gaspardo drilling machine, the rapid death of more than 200 foraging bees was reported with a clothianidin content of 0.5—11 µg/bee. Foraging bees induced to fly over the field captured at the end of sowing were reported to contain 78—1240 ng/bee clothianidin (n=5, mean = 570 ng/bee). After sowing with 2009 seed, dead bees in the field were reported to have an external clothianidin concentration of 396 ng/bee and a total concentration of 674 ng/bee. Dead bees sampled at the hive were reported to show significantly lower concentrations with external concentrations < LOD and total concentrations of 155 ng/bee at 3 hours after sowing and 119 ng/bee at 24 hours after sowing. Bees that were kept in cages at 1—9m on both the right and left side from the drilling machine were reported to have high, but variable residue levels.

No information was provided as to whether sticking agents were used in the seed treatments discussed. Also, no information was provided on whether additional "lubricants," such as talc or graphite, were used to aid in moving seed through the seeding equipment. The study does not address the uncertainty of how exhaust emissions from stationary seeders are reflective of emissions and/or potential deposition on to adjacent land compared to seeding equipment which is moving. It is also unclear how similar the European sowing machines used in this study are to seeding equipment used in the United States. Although caged bees at various locations downwind from the stationary sowing equipment were found to contain elevated residues, the study does not provide sufficient information to determine how reflective this exposure scenario is of actual conditions. Also, while free foraging bees were conditioned to fly in close proximity to seeding equipment, it is uncertain as to the likelihood that bees would naturally be attracted to a field that is being seeded and the extent to which they would encounter exhaust. Therefore, EFED cannot find that this scenario is representative of real-world conditions. Given these limitations, the exposure data on exhaust emissions would likely be considered of medium qualitative use for clothianidin risk assessment purposes.

L. EPA's incident documentation is inadequate to provide full picture of CCD-related mortalities.

EFED Response: EPA acknowledges that the information contained in the Agency's incident databases cannot capture every bee-related mortality incident. The Agency is

seeking ways to facilitate beekeepers reporting bee kill incidents using the National Pesticide Information Center (NPIC) incident reporting portal http://pi.ace.orst.edu/erep//. *However, the pesticide residue analyses from national surveys of commercial honey bee colonies indicate that neonicotinoids are detected relatively infrequently in migratory colonies and that colonies which were eventually determined to have succumbed to CCD did not contain elevated levels of neonicotinoids including clothianidin. While surveys of stationary colonies have resulted in more frequent detections of neonicotinoids and at higher levels than those reported for migratory colonies, these surveys were associated with colonies primarily in residential settings and these colonies did not exhibit CCD.*

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